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Project Final Report

"Interaction of Inorganic Nanoparticles with Cell Membranes."

EUROPEAN OFFICE OF AEROSPACE RESEARCH AND DEVELOPMENT, LONDON

Contract Number: FA8655-07-M-4007
Period: 1. 4.2007 – 30. 06. 2008

1. Introduction

The discussion regarding toxic effects of nanoparticles, especially for people exposed to the particles during manufacturing, use of nanomaterials or because the particles have entered the biosphere at the end of the life cycle, show that there is very little information regarding the interaction of these particles with living systems. On the other hand, the same knowledge is necessary to develop nanosized particles for biomedical applications. In this project we tried to start of a successful cooperation combining the unique knowledge of the participating research groups in the field of colloidal and biological behaviour of nanoparticles. Questions regarding the colloidal behavior of particles in biological liquids (blood, body liquid, and media for cell cultures) as well as concerning the up-take mechanism and the behavior of the particles inside the cells are the subject of the project. During this work, we have discovered, that superparamagnetic particles specifically target to organelles show very specific protein adsorption which enables us understand better the pathway of our particles through the membrane and inside the cell. Combined with investigation regarding the protein adsorption and their influence on the colloidal stability we have now the tools to investigate and perhaps to understand better the behaviour of nanoparticles in living systems.

2. Research work

During the preparation phase of this project we have defined following important questions regarding the investigation of the interaction of inorganic nanoparticles with cells:

- How influences the colloidal stability (agglomeration of the nanoparticles) the up-take mechanism?

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From – To)*
20-10-2008	Final Report	23 April 2007 - 23-Apr-08

4. TITLE AND SUBTITLE

Interaction of inorganic nanoparticles with cell membranes

5a. CONTRACT NUMBER
FA8655-07-M-4007

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Professor Heinrich Hofmann

5d. PROJECT NUMBER

5d. TASK NUMBER

5e. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Swiss Federal Institute of Technology
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Lausanne CH-1015
Switzerland

8. PERFORMING ORGANIZATION REPORT NUMBER

N/A

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

EOARD
Unit 4515 BOX 14
APO AE 09421

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
SPC 07-4007

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15.

The discussion regarding toxic effects of nanoparticles, especially for people exposed to the particles during manufacturing, use of nanomaterials or because the particles have entered the biosphere at the end of the life cycle, show that there is very little information regarding the interaction of these particles with living systems. On the other hand, the same knowledge is necessary to develop nanosized particles for biomedical applications. In this project we tried to start of a successful cooperation combining the unique knowledge of the participating researgroups in the field of colloidal and biological behaviour of nanoparticles. Questions regarding the
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nanoparticles in living systems.

15. SUBJECT TERMS

EOARD, Biochemistry, Toxicology, Nanoparticles

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18, NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			TAMMY SAVOIE, Lt Col, USAF
UNCLAS	UNCLAS	UNCLAS	UL	16	19b. TELEPHONE NUMBER *(Include area code)* +44 (0)1895 616459

- Which proteins or other molecules present in cell media, extracellular liquid or in the cytoplasm adsorbed at the particle surfaces and how this adsorption influences the particle-cell interaction.

Even these questions are fundamental to understand the toxic behavior of particles they are still not answered in a satisfied manner. Therefore, LTP has started in the framework of three different projects (Swiss National Foundation of Science, EOARD project, Competence Center of Materials Science Switzerland) research work aiming

1. the synthesis of superparamagnetic iron oxide nanoparticles with a controlled coating and functionalization with proteins. The proteins have to adsorb specifically at organelles or membranes of cells
2. development of methods which allows the controlled up-take of such coated particles and after a incubation time of several hours the re-separation of the particles in a magnetic field and the analysis of the adsorbed proteins
3. development of quantitative investigation of the colloidal behaviour of the particles in different biological liquids.

The collaborator paid from the EOARD grant was involved especially in the topics 2 and 3. He built-up the cell–lab and carried out the up-take experiments, the separation of the superparamagnetic particles from the cell and the measurement regarding the colloidal stability of nanoparticles.

3. Results

3.1 Protein adsorption and detection

Mitochondria play a crucial role in cellular metabolism and apoptosis and are therefore interesting targets for superparamagnetic iron oxide particles (SPION) coated with microchondria targeting peptides.
Peptide and fluorophore surface derivitization of aminopropyl triethoxysilane (APS) coated superparamagnetic iron oxide nanoparticles (APS-SPIONs) were accomplished in a fixed bed reactor using methods developed in our lab[1]. Coupling of the peptide(s) and fluorophore on the magnetic particles was accomplished using a heterobifunctional polyethylene glycol (PEG) cross linker. Three different nanoparticle groups were manufactured. 7-Hydroxycoumarin-labelled SPIONs were used as is, or coupled with either the mitochondrial targeting peptide (MTPSPIONs), or cyclic RGD in parallel with the mitochondrial targeting peptide (MTP-cRGDSPIONs). The cyclic pentapeptide cRGD (containing Arg-Gly-Asp motif) is well known to bind the V3 integrin, a transmembrane heterodimeric glycoprotein complex that is highly expressed at the surface of activated endothelial cells during angiogenesis and in tumors. The mitochondrial targeting peptide we employed is an N-terminal 20 residue sequence from the protein mitochondrial 3-oxoacyl-Coenzyme A thiolase. The peptide we synthesized contains the sequence MALLRGVFIVAAKRTPFGAYGC, where the GC residues were engineered into the

[1] 4. Steitz, B. et al. Fixed bed reactor for solid phase surface derivatization of superparamagnetic nanoparticles. *Bioconjugate Chem.* **18,** 1684-1690 (2007).

peptide to allow for a spacer (glycine) and a C-terminal cysteine for facile conjugation to the functionalized SPIONs (Details see Fig 1).

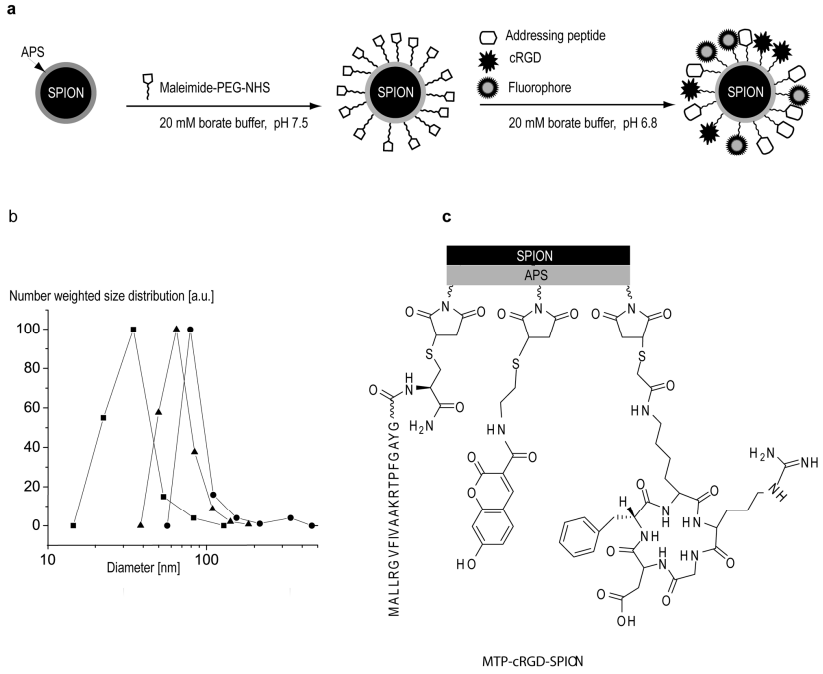


Figure 1: Surface derivatization and characterization of mitochondrial targeted APS-SPIONs. (a) Schematic representation of the two-step surface functionalization of APS-SPIONs. (b) Photon correlation spectroscopy number weighted hydrodynamic size distribution of subsequently functionalized SPIONs. ■ APS-SPION, ▲ MTP-SPION, • MTP-cRGD-SPION;

We observed the initial zeta-potential of 33.3 ± 0.9 mV for APS-SPIONs to decrease significantly after coupling to the heterobifunctional PEG to -20.8 ± 0.6 mV. Subsequent peptide derivatization resulted in a further change of zeta potentials to -10.6 ± 0.4 mV, -9.4 ± 0.4 mV, and -13.9 ± 0.4 mV for cRGD-SPION, MTP-SPION and MTP-cRGD-SPION, respectively. The mean particle size (hydrodynamic diameter) increased from 23.8 ± 0.6 nm for APS-SPIONs to 64.5 ± 0.6 nm for MTPSPIONs and 76.9 ± 0.5 nm for MTP-cRGD-SPIONs.

HeLa cells (a human cervix carcinoma cell line) were cultured and incubated in the presence of the three SPION nanoparticle preparations mentioned above. The iron content in control samples without SPIONs measured below the detection limit of the assay. After 2 hr, APS-SPIONs were almost quantitatively taken up into the cells, whereas the peptide derivatized SPIONs showed significantly lower cellular uptake. The MTP-cRGD-SPIONs internalization increased after 6 hr, at which time the differences between the three groups were most prominent. After 24 hr incubation, the uptake of functionalized SPIONs by the HeLa cells was similar in all three groups, indicating that phagocytosis had become the predominant mechanism of particle uptake, potentially due to agglomeration (Fig 2).
For analysis of the APS-SPIONs after incubation with MTP-cRGD-SPIONs, the cultures were disintegrated and the nanoparticles were recovered via magnetic separation from the whole cell lysate (Figure 3). Non-binding and only weakly binding proteins and other components were

removed by washing. To identify physiological biomolecular interaction partners, the recovered proteins were analyzed by separation using SDS-PAGE followed by in-gel tryptic digestion and identification using ESI+LC-MS/MS (Figure 4).

In combination with the fluorescence images, the mass spectrometry data confirms that targeted nanoparticles are actively transported to the mitochondria using the same pathway as the fused posttranslational sequence directs the import of the mitochondrial 3-oxoacyl-Coenzyme A thiolase[2]. Therefore the targeting peptide must have remained bound to the SPIONs and was able to actively direct the transport of the material to the mitochondria.
Analysis of the APS-SPIONs, without mitochondrial targeting, resulted in the identification of albumins, keratins and actins (data not shown) indicating that these particles may adsorb serum proteins prior to cell uptake as well as interact with proteins within the cell after endocytosis. When cell lysate instead of living cells were incubated with MTP-cRGD-SPIONS, no specific proteins could be identified.
The proteins identified interacting with the MTP-cRGD-SPIONs using mass spectrometry were further correlated to known cellular interaction pathways using the STRING database21. We found that 48 proteins formed a network that consisted of 308 interactions (Figure 5). cRGD-SPIONs showed merely unspecific interactions in the cell and 74 proteins consisting of 461 interactions were identified. cRGD-SPIONs demonstrated low affinity towards mitochondria and more important interaction with nucleus proteins compared to MTP-cRGD-SPIONs. Detailed MS results are shown in the Supporting Information.
Our data has confirmed the presence of annexin-I (ANX1) and annexin–II (ANX2), which have been reported to play a role in regulating traffic within the endocytotic pathway[3]. In addition, nucleolin (NUC) was also identified. This protein is reported to be localized in the plasma membrane and is involved in the cellular uptake of peptides rich in basic amino acids[4]. Translocation of the particles toward the mitochondria most likely occurred by an ATP-dependent interaction of the immobilized MTP with multiple Hsp70/Hsp90 (HSPA2, HSPA5-8/HSP90AA1, HSP90AB1) complexes[5]. Mitochondrial chaperonin Hsp60 (HSPD1) and cytoplasmic actin 2 (ACTB) both display 19 direct interaction partners (Figure 3). From our data, the identified chaperonin TCP complex (CCT2 and CCT6A) represents a link between the central transport complexes, which are Hsp60 and the cytoskeletal constituents.

The combination of functionalized SPIONs and their ability to be recovered using a magnetic column coupled with biomolecular mass spectrometry has allowed us to explore a complex intracellular pathway using a peptide that is known to target the mitochondria. The resulting data reveals protein interaction partners that take the nanoparticles from the extracellular space to their final destination within the mitochondria. Here we demonstrated the concept of biomolecular interaction network elucicdation with an organelle-targeting peptide, but the concept would also be applicable with more specific biomolecular, rather than organelle, targeting. Future applications of this approach could also include organelle specific drug

[2] H. Arakawa, Y. Amaya, M. Mori, *J. Biochem*. **1990**, *164*, 160-164
[3] C. E. Futter, I. J. White, *Traffic* **2007**, *8*, 951-958.
[4] S. Christian, J. Pilch, M. E. Akerman, K. Porkka, P. Laakkonen, E. Ruoslahti, *J. Cell Biol.* **2003**, *163*, 871-878.
[5] D. Picard, Cell. Mol. Life Sci. 2002, 59, 1640 – 1648

delivery, gene therapy, and *in vivo* imaging for diagnostic purposes. (For more details see attached pubblication[6]

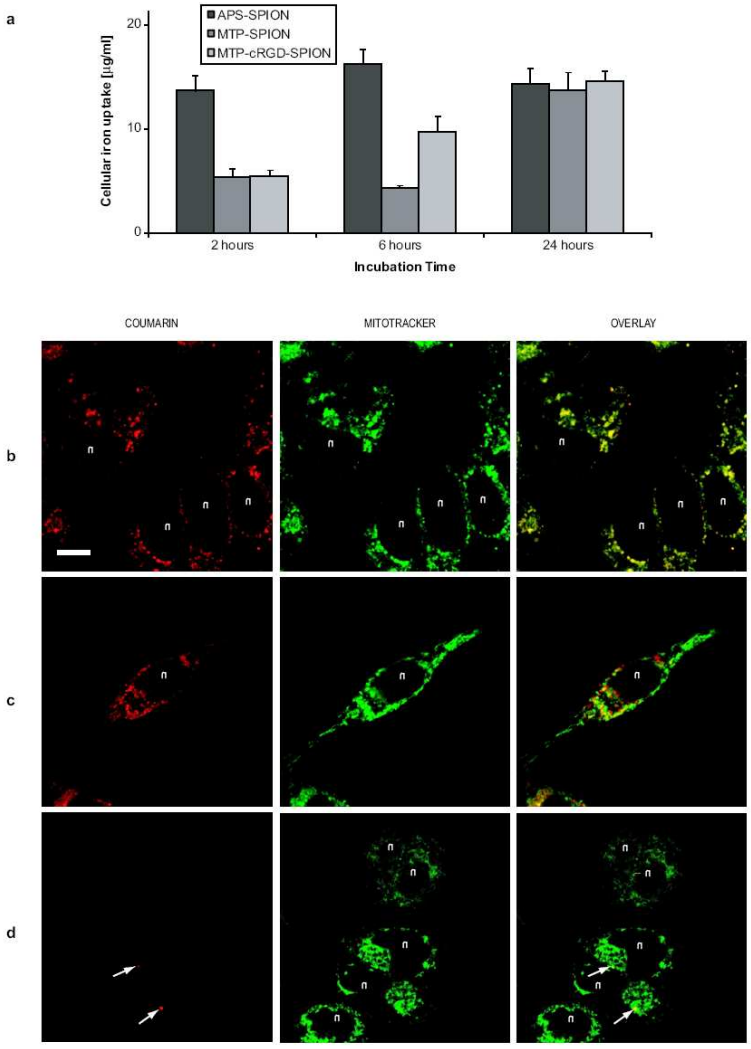


Figure 2: In-vitro uptake of SPIONs and colocalization with mitochondria (a) Cellular uptake of functionalized SPIONs over time. (b,c, and d) Confocal microscopy images of internalized SPIONs: (b) MTP-cRGD-SPIONs (c) cRGD-SPIONs (d) MTP-SPIONs.
The fluorescent signal of hydroxycoumarin is in the red channel and the Mitotracker signal is displayed in the green channel. The overlay is obtained.

[6] Jatuporn Salaklang[#], Benedikt Steitz[#], Andrija Finka[#], Conlin P. O'Neil, Marc Moniatte, André J. van der Vlies, Todd D. Giorgio, Heinrich Hofmann, Jeffrey A. Hubbell, Alke Petri-Fink Superparamagnetic Nanoparticles as a Powerful Systems Biology *Characterization Tool in the Physiological Context Angew. Chem. Int. Ed. 2008, 47, 7857-7860*

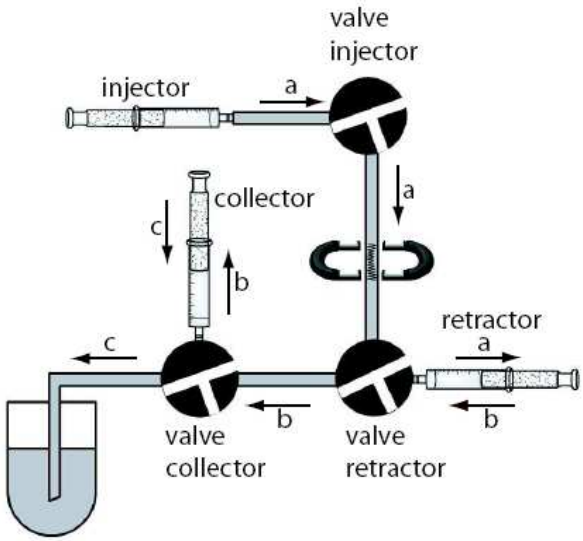


Figure 3 : Magnetic bioseparator.
The sample is first introduced into the reactor via the injector valve and magnetically immobilized for 30 minutes. After washing, the lysate is retained on the reactor by switching the valves so that the retractor can then push the washing fractions into the collector. To collect the samples after each KCl addition, the salt solution is introduced as above, and the eluted proteins are collected by switching from the retractor valve to the collection syringe. The recirculating pump is located between the injection and retractor valves.

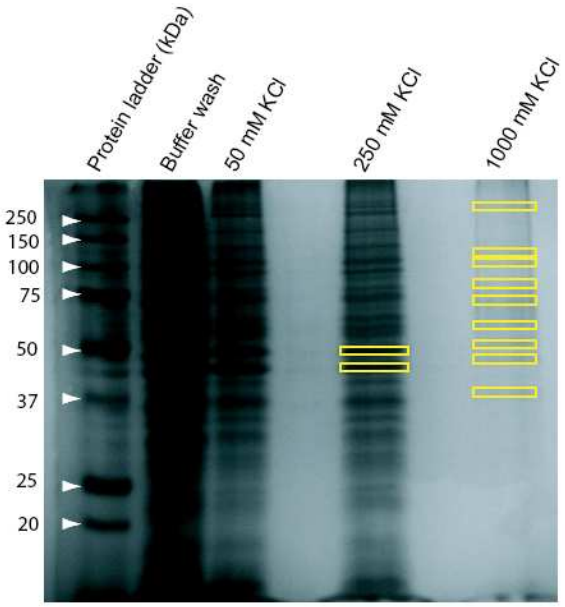


Figure 4 : SDS-PAGE analysis of eluted protein fractions from the magnetic bioseparator. Yellow rectangles represent the bands that were excised and subjected to in-gel tryptic digest and analyzed by mass spectrometry.

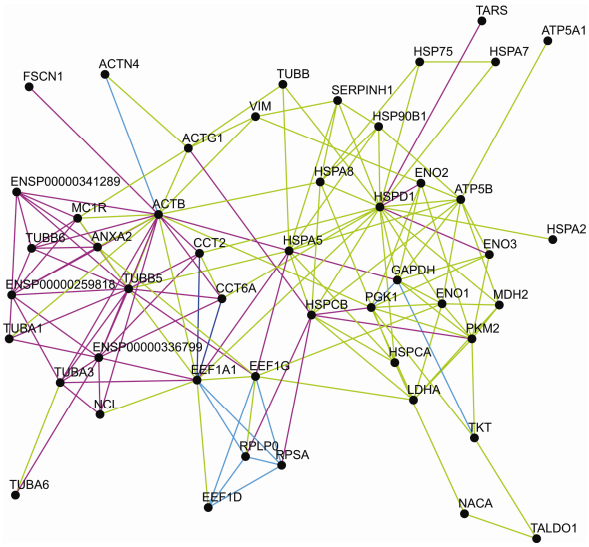


Figure 5: Evidence view of the protein interaction network in STRING. Different line colors represent the types of evidence for the association. Green: neighborhood, red: gene fusion, blue: co-occurrence, brown: co-expression, magenta: experiments, light blue: databases, and light green: text mining.

Colloidal behaviour

We have investigated the colloidal stability of different coated nanoparticles in different cell media. After the above reported results, it was clear that we have to try to analyse the adsorption behaviour of the proteins normally included in the cell media on the particle surface and there influence on the colloidal stability. It is well known that only particles between 10 and 150 (ev 200) nm will be taken up by the cells. Therefore, agglomerates of nanoparticls larger than 200 nm are not of interest for our investigation and will also have a limited impact regarding toxicity. (Our partner Dr. Saber Hussain, AFRL/HEPB, Dayton OH has just published similar observations).

The aim of this part of the project is to show in detail the influence of the composition of the polymer coating and surface charge of nanoparticles on the colloidal stability of these particles in different cell media. Furthermore, we tried to establish correlations between cytotoxicity or uptake rates and agglomeration behaviour of the particles. This correlation in particular is very important for the design of simplified toxicity tests and for the further development of such particles for *in vivo* applications.

3.2.1 Superparamagnetic nanoparticles with PVA coating:[7]

The colloidal stability of coated particles in different environments was investigated by turbidity measurements. Therefore, the particle dispersions were mixed with commonly used cell media

[7] Superparamagnetic iron oxide nanoparticles (SPIONs) were prepared by alkaline co-precipitation of ferric and ferrous chlorides in aqueous solution. The obtained brown suspension was dialyzed against 0.01 M nitric acid for 2 days, and stored at 4 ℃.
In order to obtain SPIONs coated with either polyvinyl alcohol (Mowiol® 3-83) or with a mixture of polyvinyl alcohol (Mowiol® 3-83) and vinyl alcohol/vinyl amine copolymer (M12, vinyl alcohol/vinyl alcohol copolymer mass ratio = 45) the nanoparticle dispersion was mixed at various ratios with the different polymer solutions. The products will be referred to as PVA-SPION and A-PVA-SPION in this work. For PEI coating the iron oxide nanoparticles were mixed at a PEI:Fe mass ratio of two (R=2) with 25 kDa polyethylenimine (Aldrich). The samples will be referred to as PEI-SPION in this work. DNA-PEI-SPIONs were prepared at a N/P ratio (ratio of nitrogen-containing groups of the polymer to phosphate groups of the nucleic acid) of 7.5, assuming the DNA was entirely complexed. The iron content of the suspensions was determined by redox-titration.

such as RPMI and DMEM, both in presence and absence of 10 % fetal calf serum (FCS) thereby setting the iron concentration to 100 μg /ml. After rapid homogenization, the turbidity (s) was measured by light adsorption at a wavelength of 500 nm as a function of time (t). HELA (human cervix carcinoma cells) cells were grown in RPMI medium (Gibco-BRL), supplemented with 10% fetal calf serum (FCS; Promochem) and 1% penicillin/streptomycin. One day prior to experiments, the cells were detached in trypsin-EDTA (Gibco-BRL) and grown in complete medium in 48-well plates (Costar) at ~10^4 cells per well. On the day of experiment, cells are washed with PBS and medium was changed as it was indicated. The dilutions of PVA-SPION, A-PVA-SPION, PEI-SPION and DNA-PEI-SPION were added for the concentration, time and temperature indicated. After 22 hours of incubations, the MTT assay was performed to determine cell viability. MTT reduction was used to quantify metabolically active cells.

In our study we found that A-PVA-SPIONs and PVA-SPIONs showed no important agglomeration in none of the investigated media after 30 minutes (Figure 6). Therefore, biological evaluations can only neglect the influence of the media on colloidal stability when working in this time frame. At least in the case of HELA cells, 30 minutes of incubation at the investigated concentrations, is not sufficient to achieve considerable uptake. This can be overcome by actually exploiting the superparamagnetic properties of the core iron oxide nanoparticles. In this case, uptake can be significantly enhanced in the presence of a magnetic field. After one hour of incubation significant differences between A-PVA-SPIONs and PVA-SPIONs were detected in depending on the medium. The high stability of PVA-SPIONs in FCS supplemented media could be explained by depletion stabilization caused by the serum proteins showing a similar size as the nanoparticles. Increased stability of A-PVA-SPIONs in FCS supplemented DMEM medium (after one hour) is attributed to a similar mechanism proposing the additional adsorption of small negatively charged molecules at the surface of the particles leading to a neutral surface charge as observed with PVA-SPIONs. In the case of RPMI no such preferred adsorption could take place and the adsorption of FCS on the particles could be possible, since the surface of proteins is heterogeneous with regard to charged and hydrophobic domains. If one or more FCS proteins are attached to the particles, no depletion stabilization is possible and agglomeration occurs. However, the amine content is low (compared to e.g. PEI) and this “medium” effect is overcome by the intrinsic particle properties after two hours. Already after two hours results between the two systems were comparable. Only DMEM with FCS offered long term colloidal stability for both systems. The medium and the surface of the particles have to be evaluated fundamentally using statistical methods to gain an insight into the impact and participation of both effects. The difference in agglomeration behaviour in the different media is hard to explain. The two media RPMI 1640 and DMEM vary in their glucose content which is in RPMI 1640, 2000 mg/ml, and in DMEM, 4500 mg/ml. Apart from differences in glucose content the main differences lie in the presence/absence of e.g. sodium pyruvate pyridoxine, L-arginine, L-proline and L-histidine. However, these variations could not explain the different agglomeration behaviour. The biological evaluations is still ongoing.

Fig. 7 summarizes the influences of the varied parameters (polymer, medium, and serum) on the agglomeration rate and the cell uptake. In particular, Fig. 7(a)–(c) describes the uptake rate versus the agglomeration rate at ways within one category of the investigated parameters.
For example, Fig. 7(a) shows the uptake versus the agglomeration rate for both investigated particle systems i.e., PVA-SPIONs and A-PVA-SPIONs. The combination of the assigned labels

of an identical point from Fig. 7(a)–(c) yields the experimental conditions at which the experiments were conducted. All three graphs show that the systematic variation of any of the parameters including the change of polymer [Fig. 7(a)] or medium [Fig. 7(b)] and addition of serum [Fig. 7(c)] had an observable influence on agglomeration and uptake with the same trend. The determination of the correlation between agglomeration rate and uptake yielded a coefficient of 0.73 . Replacement of PVA by A-PVA led to significantly faster agglomeration (Fig. 7(d),) and slightly higher uptake [Fig. 7(g)]. However, the impact of the polymer on cellular uptake, at least in this case, was statistically not significant . The same was observed for a medium change from DMEM to RPMI [Fig. 7(e) and (h)].

The most apparent effect was observed upon addition of serum, which divided the samples into a fast agglomerating (serum-free) and a slowly agglomerating (with serum) group (Fig. 7(f),). In contrast to the results obtained by changing the polymer or the medium, the addition of serum had a significant impact on the cellular uptake (Fig. 7(i),). There was virtually no cellular uptake observed for measurements in serum.

3.2.2 Development of Chitosan coated SPIONs for bio application

Analogue to the manufacturing of PVA and amino-PVA coated SPION, we have also synthesised chitosan coated particles as well as beads with SPION in a chitosan matrix. The reason for this additional polymer was, that chitosan is very biocompatible and is used for drug encapsulation. Regarding the difficulty to understand colloidal behaviour of PVA coated SPION, we hope that chitosan coating could bring some new information and finally explanations. Typical chitosan coated particles and beads are shown in Fig 8, the mean size and Zeta-potential is given in table 1.

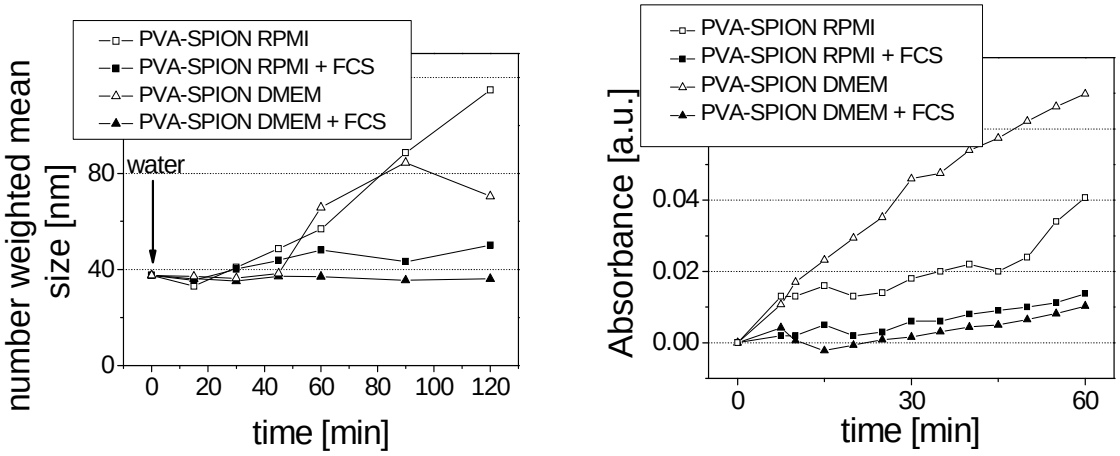
Table 1: Mean bead size and Zeta-potential of Chitosan/SPION beads

	Average (±SD*)	Zeta potential (mV) (±SD)
SPIONs	10 (±0.56)	25.3 (±1.9)
Chitosan nanoparticles	140 (±2.17)	22 (±0.3)
Chitosan-SPIONs nanoparticles	112 (±0.24)	27.5 (±1.14)
Chitosan/PEI nanoparticles	211 (±1.4)	28 (±1.5)
Chitosan/PEI-SPIONs nanoparticles	185 (±0.7)	27.6 (±0.14)

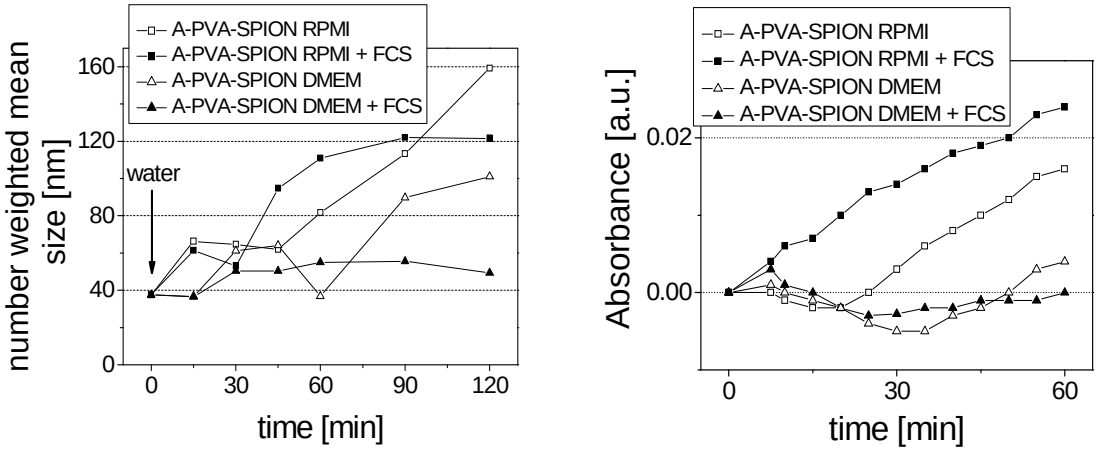
* = the mean value for three replicate measurements

A B

PVA-SPIONs



A-PVA-SPIONs



PEI-SPIONs

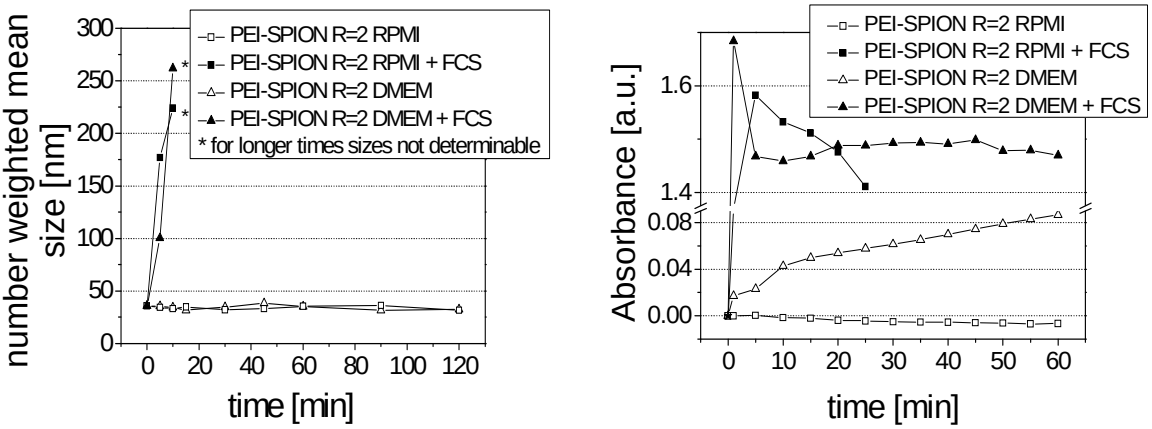


Figure 6: Stability of PVA-SPIONs, A-PVA-SPIONs and PEI-SPIONs in different cell media monitored by A) PCS measurements and B) UV measurements. The error is not indicated to simplify matters

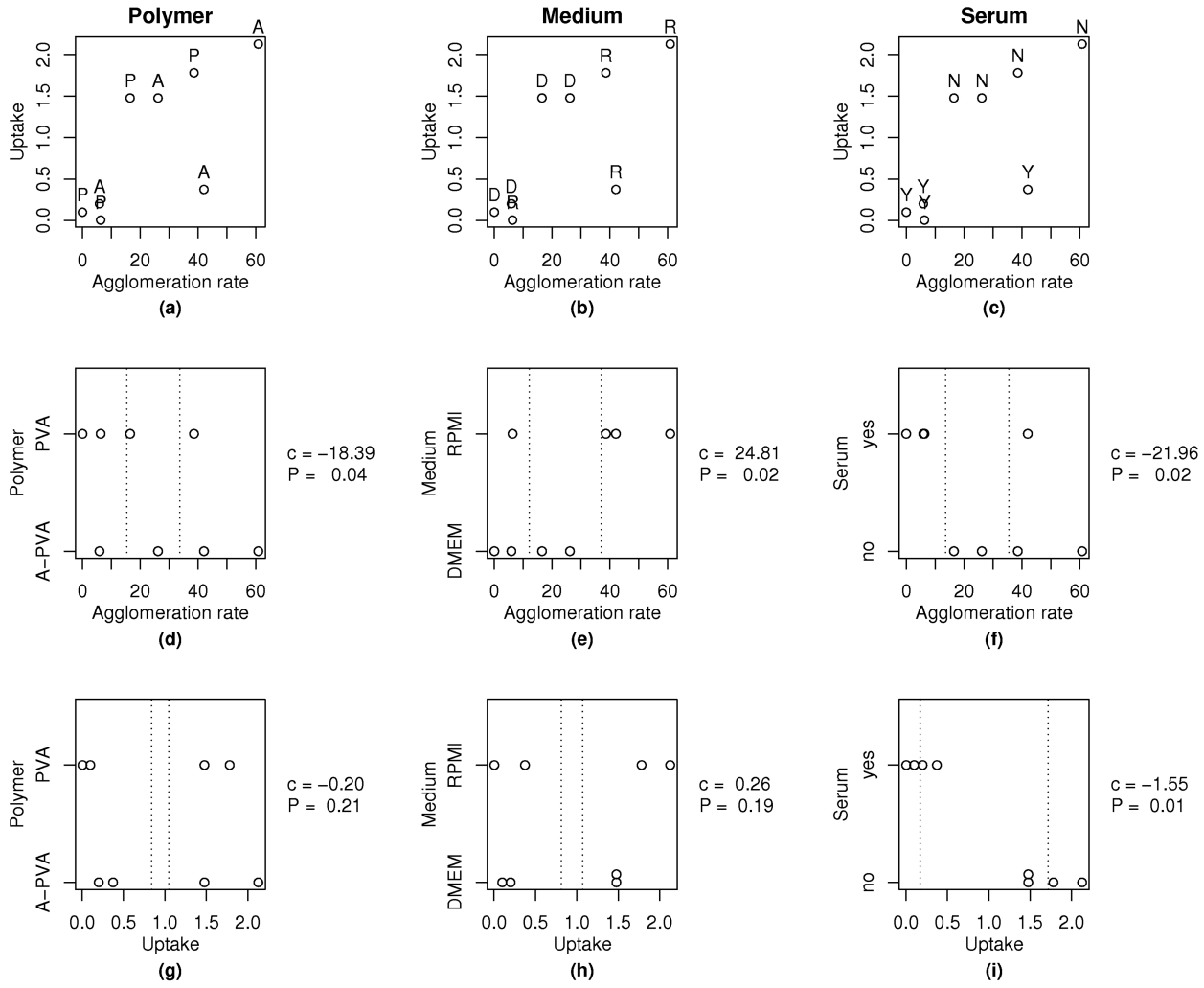


Figure 7. Influence of variations in the system. (a)–(c): Uptake versus agglomeration rate [nm/h] for the eight measurements, where in (a) labels represent the polymer (P = PVA; A = A-PVA), in (b) the medium (D = DMEM; R = RMPI), and in (c) the presence of serum (Y = serum;N = no serum). Figures (d)–(f) show strip charts, highlighting the differences in the agglomeration rate due to (d) the polymer, (e) the medium, and (f) the presence of serum. (g)–(i) show stripcharts accordingly, highlighting the differences in uptake. In each strip chart c is the coefficient of the corresponding explanatory variable in the multiple regression model. The p-value is obtained from a t-test against the null hypothesis H_o: c = 0. The mean value for each class is marked by the two dotted vertical lines.

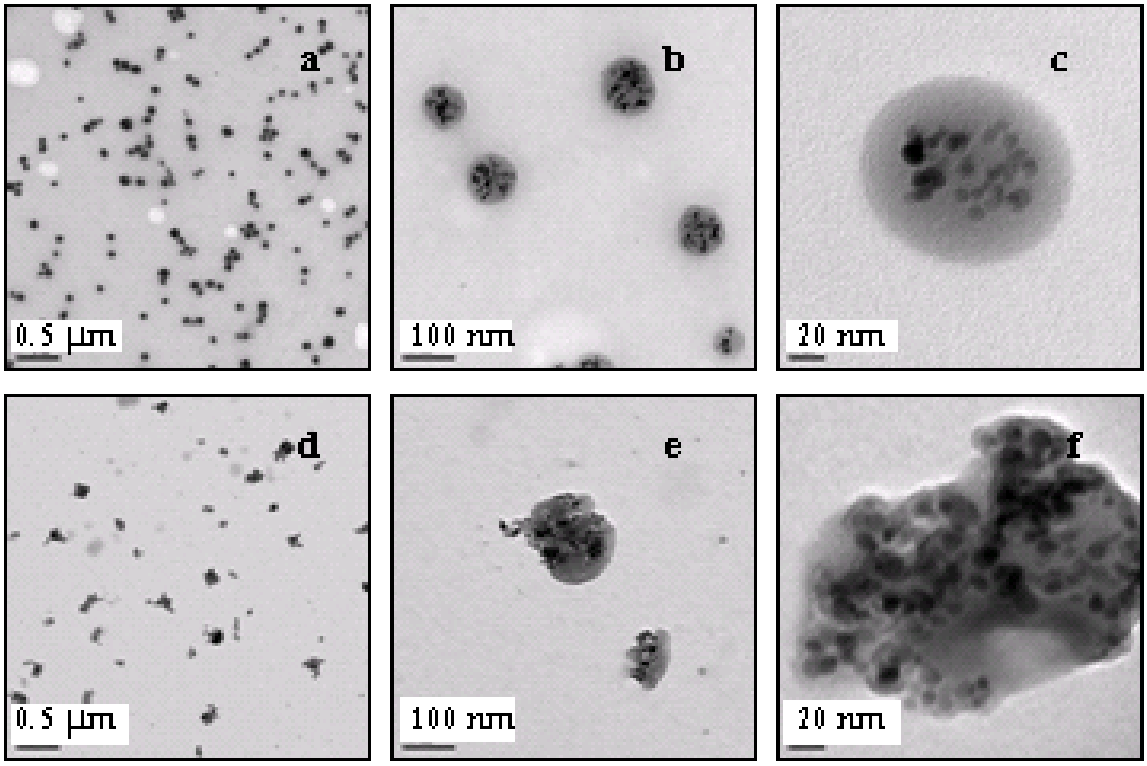


Figure 8: Typical morphology of Chitosan /SPION beads

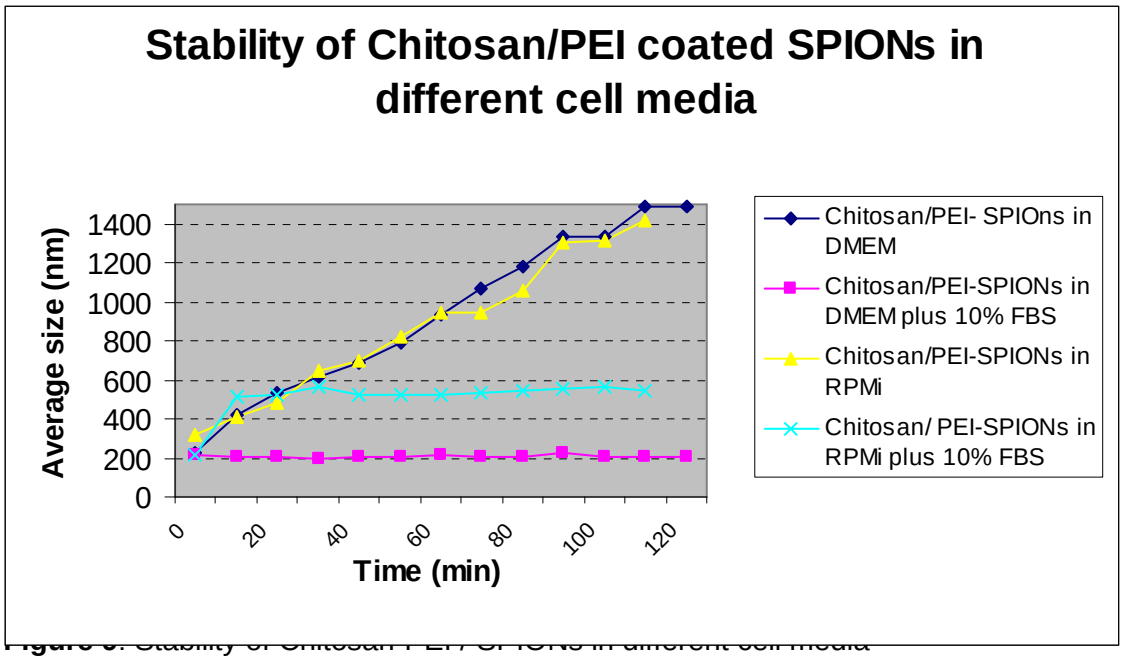
It is important to note, that the bead size is still in the interesting range of 100 to 200 nm where an up-take by cells is possible. Also of interest is that the Zeta-potential is with 25 mV slightly positive and in the range where cell up-take is normally observed. The investigated biopolymer coated SPIONs were absolutely stable in water and in PBS for a week of observation without any sign of agglomeration.

The agglomeration behaviour of Chitosan/PEI coated SPIONs in two different cell media DMEM and RPMI, both pure and supplemented with FCS, were examined by photon correlation spectroscopy (Figure 9). It was shown that the systematic variation of any of the parameters including the change of Chitosan/PEI, or medium, and addition of serum had an observable influence on agglomeration and uptake with the same trend. The addition of PEI into the reaction led to significantly higher colloidal stability both in PBS and in cell culture media. The impact of the particle surface was also observed for a medium change from DMEM to RPMI. The most apparent effect was observed upon addition of serum, which divided the samples into a fast agglomerating (serum-free) and a slowly agglomerating (with serum) group. It is interestingly to not, that in contrast to the observation with PVA/PEI coated SPION, the addition of serum to Chitosan/PEI/SPION leads to a strong agglomeration. To understand the behaviour, we have also measured the Zeta-potential of the SPION beads in contact with the cell media (Table 2). The results show clearly a very low Zeta-potential in the range of +/- 5 mV, values which are for sure too low for an electrostatic stabilisation of the particles. The change of the

Zeta-potential indicates also a strong adsorption of other molecules at the particle surface. Without serum a slightly positive and with serum a slightly negative value was observed.

Table 2: Zeta-potential of Chitosan/PEI/SPION beads in water and different cell media

Particle Typ	**Zeta potential (mV)**
Chito/PEI/SPION in water	27.8
Chito/PEI/SPION in DMEM without FBS	1.68
Chito/SPION/PEI in DMEM plus FBS	-5.83
Chito/PEI/SPION in RPMi without FBS	2.94
Chitosan/PEI/SPION in RMPi plus FBS	-5.23



To explain the change in the Zeta-potential of the beads we studied the adsorption of protein on Chitosan coated SPIONs. The particles were incubated in DMEM supplemented FCS for 15 min, 1, 3 and 6 hours. The particles after incubation with cell media were then subjected to run SDS-PAGE and coomasie staining.

Figure 10 shows the result of SDS-PAGE of proteins attached on the particle surface after the incubation. Three major proteins were found that are albumin at MW of ~60 KDa, hemoglobin A at ~ 66 KDa and IgG at~ 160 KDa when compared to the literature. However, the result needs

to be further confirmed by LC/MS/MS technique. We assume that the amount of proteins during the first 60 min is increasing and that all 3 proteins are adsorbed at the surface from the beginning. We can not explain at the moment the influence of these adsorbed proteins on the charge of the particles.

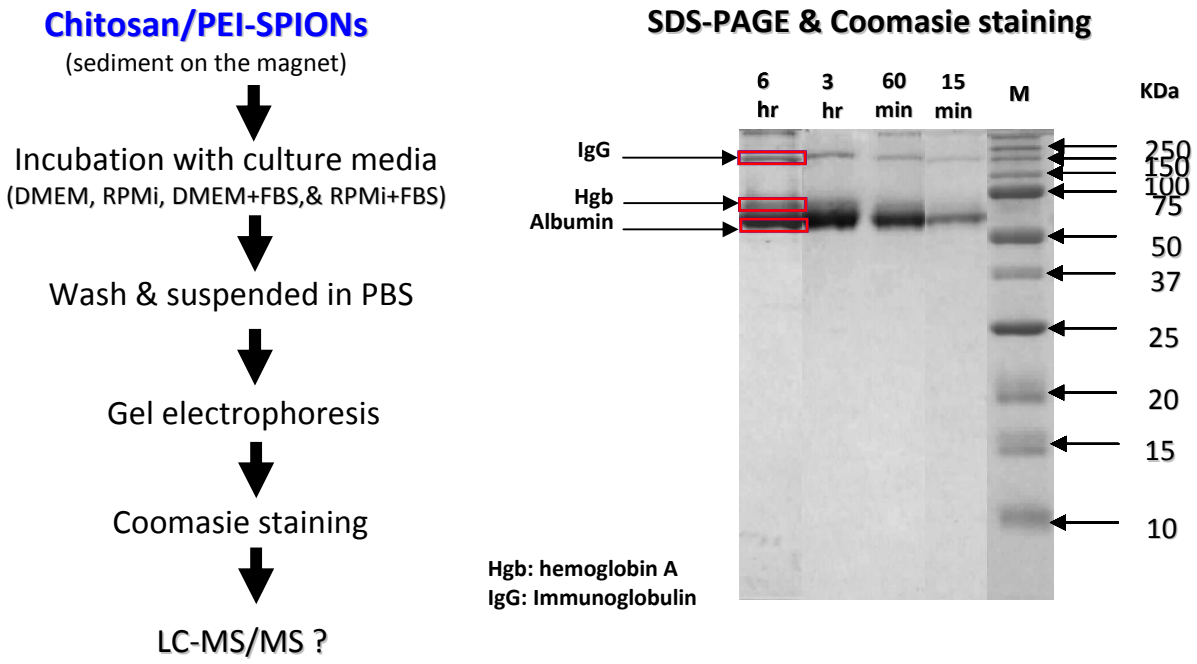


Figure 10: SDS-PAGE of proteins adsorbed on Chitosan/PEI-SPIONs

4. Contacts with US- research group

Most of the above presented results are presented during a visit at the AFRL/HEPB December 6th 2007. (Contact person: Dr. Saber Hussain, AFRL/HEPB, Applied Biotechnology; Air Force Research Laboratory/HEPB, Wright Patterson Air Force Base, AFB, OH- 45433-5707). During this meeting, further collaboration including exchange of post doc was discussed. A visit of Dr Saber September 2008 in Switzerland was also in preparation but unfortunately we were not able to realize it. It is foreseen for the coming months. The goal of that visit will be to establish cooperation based on National Science Foundation Projects., especially in the Framework of the new Swiss National Research Program "Changes and Risk of Nanotechnology" which is focussed on the toxicology of nanoparticles.

5. Impact

The presented work and the results enable us to apply interesting project in Switzerland, for example National Science Foundation (AFRL/HEPB as international partner) or in the new

Research program System-X, a Swiss wide research in System biology including particle-cell interaction. Additionally, we were able to ably for 2 EU projects in the Framework program 7.

Lausanne, October 10th, 2008

Prof. H. Hofmann
Director of the Powder Technology Laboratory